behavior toward two substrates led to the hypothesis that papain and other papainases are constituted of two partial enzymes which are inactive when associated with each other, but which dissociate in the presence of activators and thus become active.[4]

The experimental basis of this hypothesis seemed unsatisfactory because of the heterogeneous nature of albumin peptone which was employed as one of the substrates. This peptone is a mixture of various protein split-products and frequently contains substances which may influence the activity of the enzyme. We have now found that data similar to those obtained with albumin peptone can be gotten with a well-crystallized synthetic substance, benzoyl arginine amide. The hydrolysis of this synthetic compound is activated by the addition of phenylhydrazine. However, this activation is observed only if the papain preparation is a natural, unpurified extract which contains SH compounds as natural activators. Addition of phenylhydrazine to purified papain, in which these accompanying activators are absent, produces no activation towards either albumin peptone or benzoyl arginine amide. Addition of HCN or a similar activator to the purified papain is necessary to restore the activation by phenylhydrazine (Table I). A similar effect was

TABLE I
ACTIVATION OF PAPAIN BY VARIOUS REAGENTS

Enzyme preparation	Substrate		
	Albumin peptone	Benzoyl arginine amide	Carbobenzoxyisoglutamine
Natural papain	.04	.01	.09
Natural papain + phenylhydrazine	.26	.33	.05
Purified papain	.00	.02	.03
Purified papain + phenylhydrazine	−.02	−.03	.03
Purified papain + phenylhydrazine + HCN	.65	.16	.01
Purified papain + phenylhydrazine + cysteine		.60	.48

The splitting is expressed as the increase in cc of 0.01 N KOH per 0.2 cc of the reaction mixture. An increase of 1 cc for the synthetic compounds corresponds to 100 per cent. splitting of one peptide linkage. Time interval, 24 hours. Temperature, 40° C. pH, 5.0.

reported[5] for the splitting of carbobenzoxyisoglutamine where the presence of a large concentration of cysteine effected an activation of purified papain + phenylhydrazine. These experiments tend to show that in the behavior of papain toward various substrates there is no absolute differentiation but rather a relative one which depends upon the nature of the substrate. In view of this finding, the hypothesis of a two-enzyme system in papain becomes at present superfluous.

[4] M. Bergmann and J. S. Fruton, SCIENCE, 84: 2169, 1936.

[5] M. Bergmann, J. S. Fruton and H. Fraenkel-Conrat, *Jour. Biol. Chem.*, 119: 35, 1937.

Synthetic substrates were also used to study the effect of several purification procedures on the activity of papain. It was observed that essentially all the proteolytic activity toward both gelatin and carbobenzoxyisoglutamine is precipitated between 0.5 and 0.7 saturation with respect to ammonium sulfate.[6]

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OSMOTIC EFFECTS OF DEUTERIUM OXIDE (HEAVY WATER) ON LIVING CELLS

IN considering the permeability of erythrocytes to D_2O[1] it occurred to me that D_2O, because its vapor pressure was less than that of H_2O, would cause osmosis in the same way that concentrated solutions would. The vapor pressures[2] of D_2O and H_2O were 15.06 and 17.36 mm respectively at 20°, and H_2O in a 7.43 M ideal solution would have a vapor pressure equal to that in pure D_2O, *i.e.*, 15.06 mm. In this case we might suppose that the rate of osmosis is roughly parallel to the difference of equilibrium vapor pressures, *i.e.*, fugacities of the two types of water. I have found that plasmolysis of plant cells and hemolysis of erythrocytes occurs, although osmosis is so transient that in my experiments hemolysis is incomplete, and plasmolysis is expressed only by shrinkage of cell and cell wall together.

I have studied the shrinkage preceding plasmolysis in *Nitella clavata* A. Br., using a method used by Collander and Bärlund[3] for penetration of alcohol into similar material, *Chara*. We have isolated terminal "leaves" 5–12 mm long, attached to a node, and left them in double distilled water for about a day to recover. A leaf was fixed by dropping wax (3 parts cacao, 1 beeswax) on the basal end, meantime supporting the free end with wet filter paper, in a glass cell 20 × 6 mm, and 4 mm deep. About 0.4 ml of water was put in, and the filter paper removed. The cell was put on a mechanical stage under a 16 mm objective (Bausch and Lomb) and No. 8 periplan (Leitz) oculars containing an ocular micrometer. One of the divisions of the scale of this micrometer measured 7.2 μ in the focal plane. Using 1 ml pipettes water was removed and replaced several times to make sure of the regular behavior of the leaf. When the leaf

[6] A. K. Balls, H. Lineweaver and R. R. Thompson (SCIENCE, 86: 379, 1937), used this property for the isolation of papain crystals.

[1] S. C. Brooks, *Jour. Cell. Comp. Physiol.*, 7: 163–171, 1935.

[2] G. N. Lewis and R. T. MacDonald, *Jour. Am. Chem. Soc.*, 55: 3057–59, 1933.

[3] R. Collander and H. Bärlund, *Acta bot. Fenn.*, No. 11, 1–114, 1933.

regularly came back immediately to the same zero on the micrometer after each change with no change in length, D_2O[4] was now introduced in the same way. Observations were made, and when shrinkage and recovery were completed, D_2O was replaced with H_2O and observations again made. This was repeated for D_2O and usually also for H_2O. Fig. 1 shows the

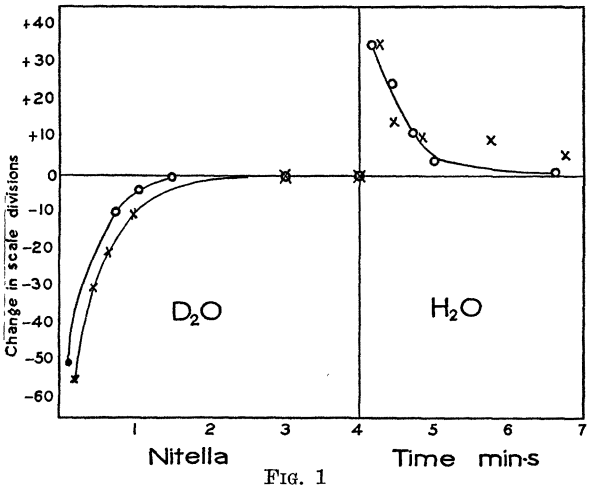

FIG. 1

changes in micrometer divisions, showing shrinkage after application of D_2O, followed by recovery (*i.e.*, swelling) to the original length, while on applying the H_2O, after D_2O, the filament first elongated and then returned to the original length. Altogether six tests for D_2O and five for H_2O were made. All experiments gave the same general behavior, but there was failure of the leaf to return to zero in some cases, either because of slipping of the attachment or the mechanical stimulation by bending against the sides of the glass cells. Observations in D_2O were made for 2 to 11 minutes, and in H_2O for 4 to 12 minutes.

The leaves of *Nitella* shrank 0.60–1.37 per cent. in D_2O, and elongated 0.40–0.70 per cent. in H_2O. These values were observed as nearly as possible at 5 or 10 seconds. The process of putting the water in the glass cell occupies 2 to 4 seconds. Even before the leaf appeared in the microscope field it had passed through a maximum, and by the time observation was made it was already returning toward zero. By this method the true maxima could not be observed, and since the whole recovery occurred in about 1 minute in D_2O, the time recorded in the earlier observations were actually only approximately measured. One notes that D_2O acts as a plasmolytic agent, although plasmolysis is concealed by the stretched elastic cell wall which follows the shrinkage. H_2O causes swelling of the cell, the counter change of that produced by D_2O.

It should be possible to make such cells as erythrocytes to swell on return to an H_2O solution from an equally concentrated D_2O solution, thus causing hemolysis. This is analogous to hemolysis caused by treating erythrocytes with hypotonic solutions, since H_2O is hypotonic to D_2O. We have tried using D_2O and H_2O solutions in 1.1 per cent. or 0.8 per cent. NaCl. Erythrocytes were obtained by centrifugation from 0.2 ml of defibrinated sheep blood. In the first experiment 1.1 per cent. NaCl solutions were used. In the second experiment 0.8 per cent. NaCl solutions were used, and the sediment was first washed in H_2O–NaCl solution by centrifugation and decantation. After that in both cases the erythrocytes were suspended in 1.0 ml of D_2O–NaCl, allowed to stand for 10 minutes, then centrifuged, and the D_2O–NaCl decanted, the decantate saved. The sediments were suspended in 5.0 ml of corresponding H_2O–NaCl solutions (1.1 and 0.8 per cent. respectively). Hemolysis was shown by the discolored supernatant fluids decanted after suspension and centrifugation. The remaining erythrocytes were hemolysed in 5.0 ml of pure H_2O. In the first experiment about 22 per cent. of the hemoglobin was in the H_2O–NaCl fluid, as compared with 78 per cent. in the fluid from the last sediment of cells. In the second experiment colorimetric measurements showed that the H_2O–NaCl solution contained 38.7 per cent. of the hemoglobin, 61.3 per cent. in the final sediment. The decanted D_2O was not perceptibly discolored.

The erythrocytes suspended in a D_2O–NaCl solution take up D_2O. These cells would have a vapor pressure much lower than that of the H_2O–NaCl, the H_2O in a few seconds enters the cells and this swelling brings hemolysis of the weaker part of the erythrocytes. Here again D_2O is hypertonic to H_2O and is an active osmotic agent.

The osmotic effects of D_2O as compared to H_2O produce some of the well-known phenomena associated with biological effects. The press, for example, mentioned the "burning sensation" experienced on drinking D_2O. Lewis[5] describes a mouse which had access to D_2O only and "the more he drank of the heavy water the thirstier he became." "The symptoms of distress that he showed seemed more pronounced after each dose, but not cumulative with succeeding doses." Since D_2O violently dehydrates living cells it is quite understandable that D_2O causes thirst and other osmotic effects.

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DISULFIDE FROM AMMONIUM SULFATE IN THE PRESENCE OF MASHED ROOT-TIPS OF PHASEOLUS VULGARIS

A NATURAL result of the finding of nearly ten years ago that the sulphydryl group and its partially oxi-

[4] D_2O was procured from the Stuart Oxygen Company. Its density was 1.1079 at 25°, and it was 99.9%+ pure.

[5] G. N. Lewis, SCIENCE, 79: 151–153, 1934.